ELSEVIER
Biochimica et Biophysica Acta 1265 (1995) 127–132
BBA Biochimica et Biophysica Acta

# Regulation of cellular pH in skeletal muscle fiber types, studied with sarcolemmal giant vesicles obtained from rat muscles

Carsten Juel *

*The Copenhagen Muscle Research Centre, August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark*

Received 26 August 1994; accepted 4 November 1994

**Abstract**

Sarcolemmal giant vesicles obtained from rat hindlimb muscles were used as a model for the study of pH regulation in skeletal muscle. The transport systems involved in the recovery from 40 mM lactate and $pH_i$ 6.5 were quantified from both flux measurements of the co-transported ions and counter-ions, and from measurements of the rate of the internal pH change. The diffusion of lactic acid plus the carrier-mediated co-transport of lactate and $H^+$ had the highest capacity to transport protons (240 nmol $H^+$/mg protein per min). These systems are therefore responsible for a large part of the $H^+$ efflux in periods with a high lactate production. The capacity of the $HCO_3^-$-dependent systems was 47 nmol/mg per min, and the capacity of the $Na^+/H^+$ exchange system was 33 nmol/mg per min in vesicles from mixed muscles. The capacity to remove $H^+$ by the lactate/$H^+$ co-transport system and by the bicarbonate-dependent systems was significantly higher in vesicles from predominantly red fibers than in vesicles from white fibers, whereas the distribution of the $Na^+/H^+$ exchange system was independent of fiber type. These observations demonstrate that the pH regulation during muscle activity in red muscles is more effective than in white muscles.

*Keywords:* Lactate/$H^+$ co-transport; $Na^+/H^+$ exchange; Bicarbonate/$Cl^-$ exchange

## 1. Introduction

In muscle cells as in most other cell types internal pH ($pH_i$) is more alkaline than predicted if $H^+$ was passively distributed. The passive influx of $H^+$, which is driven by the electrochemical gradient, must therefore be counteracted by a $H^+$ efflux mediated by one or more specific acid extruding membrane transport systems.

The transport systems responsible for the $pH_i$ regulation at rest have been studied with $CO_2$ or $NH_4^+$ prepulses and subsequent measurements of $pH_i$ recovery. The $pH_i$ recovery can be quantified with $H^+$-sensitive micro electrodes, with pH-sensitive dyes, or the pH can be calculated from the distribution of weak acids [1,8,23].

The main $pH_i$ regulatory system in resting mouse and rat soleus and diaphragm is the amiloride-sensitive $Na^+/H^+$ exchange system, whereas the DIDS-sensitive and $HCO_3^-$-dependent system plays a minor role [1,23]. Meanwhile a more active role for the $HCO_3^-$-dependent system has been reported for extensor digitorum longus muscle [8]. The pH regulation by resting muscle has been reviewed by Aickin [2]. These studies were carried out with resting muscles and can only account for the pH regulation at rest. During muscle activity both $H^+$ and lactate are formed. The lactate accumulated in muscles activated to fatigue can reach 40–50 mM in cell water, and the internal pH can decrease to approx. 6.5 [11]. During muscle activity and in the recovery period after activity the muscle therefore is facing a double problem; to eliminate the surplus of both $H^+$ and lactate ions.

Some of the accumulated lactate is metabolized, but a considerable fraction is released from the cell. The transmembrane transport of lactate in skeletal muscle is mediated mainly by a lactate-$H^+$ co-transport system and to some extent by passive diffusion of undissociated lactic acid [10,12,22,25]. Both these types of lactate effluxes are paralleled by a movement of $H^+$, and are therefore also responsible in cellular $pH_i$ regulation during and after muscle activity. This relationship has previously been shown in isolated muscles where the inhibition of lactate

* Corresponding author. E-mail: cjuel@aki.ku.dk. Fax: +45 35 321567.


*SSDI* 0167-4889(94)00209-6

transport delayed $pH_i$ recovery [10]. However, the quantitative importance of the lactate/$H^+$ co-transport system for $pH_i$ regulation in active skeletal muscle has not been established. On the contrary, it has been stated for cardiac muscle that the $Na^+/H^+$ exchange has the highest transport capacity in the sarcolemma [6], and that lactic acid efflux from skeletal muscle seems to be too slow to account for much proton efflux in recovery [15].

The purpose of the present report was to study all three acid extruding systems in the same preparation and to describe the relative importance of these transport systems in different fiber types. The capacity to remove $H^+$ was quantified from both the measurements of pH changes, and from the flux of counter-ions and co-transported ions. The experiments were carried out with sarcolemmal giant vesicles [12,20]. The advantages with this model compared to intact muscles were that both the internal and external ion composition could be controlled and that the $H^+$ and lactate distributions were not influenced by metabolism. The experiments were carried out with artificial internal acidifications and initial lactate concentrations simulating the conditions in muscle cells fatigued to exhaustion.

## 2. Materials and methods

Rat hindlimb muscles were treated with collagenase and the spontaneously formed vesicles were isolated as previously described [12]. It has been shown that the vesicular membranes are predominantly of sarcolemmal origin [19,20]. Vesicles from 'red' fibers were obtained from soleus, red gastrocnemius, vastus intermedius, and parts of mixed gastrocnemius, while white gastrocnemius, gracilis, tensor fasciae latae, vastus medialis and white vastus lateralis were used to represent 'white' fibers [3]. The giant sarcolemmal vesicles have been used in all the following procedures.

### 2.1. Lactate efflux experiments

The method for efflux measurements has previously been described [12]. In short, vesicles were loaded with [L-$^3$H]lactate, unlabelled lactate and [$^{14}$C]sucrose, the latter was used as an extravesicular marker. After 30 min incubation the vesicles were spun down with the incubation medium present. The efflux started when 10–25 $\mu$l of the sedimented vesicles were transferred to 25 ml of magnetically stirred efflux medium. In zero *trans* experiments external sucrose was used to compensate for any change in osmolality. The volume of the efflux medium was at least 1000-times the vesicular volume, therefore a back flux (uptake) of labelled lactate can be neglected. In the efflux period 10–15 vesicle free samples were obtained from the efflux medium with syringes mounted with a 0.25 $\mu$m filter. The $^3$H and $^{14}$C counts were determined with a Tri Carp liquid scintillation counter. The lactate efflux was complete within 10–12 min. Initial lactate efflux could be calculated from a multi-exponential fit to the data as previously described [12]. Carrier-mediated efflux was defined as the inhibitor-sensitive fraction of the total efflux [12,26].

In experiments with low internal pH (6.5), the vesicles were incubated twice (30 min) at the new pH value and spun down ($830 \times g$, 30 min). In some experiments the internal pH was checked with the BCECF method (see below). Protein content was determined according to Bradford [5] using bovine serum albumin as a standard.

### 2.2. $Cl^-$ efflux measurements

Vesicles were incubated twice with 140 mM KCl, 5 mM Mops, 20 mM NaCl, pH 6.5 adjusted with HCl/NaOH in order to decrease $pH_i$. $^{36}$Cl was added in the second incubation. After incubation (30 min) the vesicles were spun down (30 min), the total equilibration time for $^{36}Cl^-$ was therefore 60 min. pH measurements with the BCECF method (see below) showed that the low internal pH was obtained with this treatment. The efflux experiments were carried out as the lactate efflux experiments (see above) with two different efflux media. $HCO_3^-$ medium: 140 mM KCl, 25 mM $NaHCO_3$, equilibrated with 5% $CO_2$/95% air (pH 7.4, inward directed $HCO_3^-$ gradient 25 mM). $HCO_3^-$-free medium: 140 mM KCl, 5 mM Mops, 20 mM NaCl, pH 7.4 adjusted with HCl/NaOH. In some of the $HCO_3^-$ experiments the vesicles were preincubated in the dark with 0.2 mM DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid) for 60 min. The equilibration time for labelled $Cl^-$ was approx. 20 min. The initial $Cl^-$ efflux rate was calculated from a fit to the data.

### 2.3. Sodium influx experiments

Vesicles were preincubated in 125 mM KCl, 15 mM NaCl and 5 mM Mops/Mes at pH 6.5 or 7.4 (internal medium). 10 $\mu$l vesicles (approx. 0.27 mg protein, extravesicular space $< 5$ $\mu$l) were placed in a small test tube and incubated for 30 s with 100 $\mu$l 100 mM NaCl, $^{22}Na^+$, 40 mM KCl, 5 mM Mops at pH 7.4 (external medium). [$^3$H]sucrose was used as extravesicular marker, which allowed a correction for extravesicular $^{22}Na^+$. The 1.5 ml ice-cold stop solution contained KCl/Mops at pH 7.4 and 1 mM amiloride. Initial experiments showed that the equilibration time for labelled $Na^+$ was more than 10 min. The influx during the 30 s incubation used in the present experiments therefore represents the initial flux.

### 2.4. Measurements of internal pH

Vesicles were incubated for 60 min at room temperature with 1 $\mu$M of the membrane permeant acetomethyl ester

form of BCECF (Molecular Probes, Eugene, OR, USA), taken from a stock solution dissolved in anhydrous dimethylsulfoxide, DMSO. The vesicles were then diluted and spun down in order to remove the external probe. The internally formed and trapped impermeant BCECF, which shows pH-dependent fluorescence, was measured spectrofluorometrically (excitation 505 nm, emission 535 mm). In calibration experiments and after each experiment the protonophore CCCP (10 $\mu$M) dissolved in ethanol was used to equilibrate the external and internal $H^+$ concentrations. The internal pH was acidified either with lactic acid (if $H^+$/lactate co-transport was studied), or with ammonium chloride preincubation (if $Na^+/H^+$ was measured). The amiloride derivative EIPA (5-*N*-ethyl-*N*-isopropyl-amiloride) was used instead of amiloride which influenced the fluorescence signal. 10 or 15 $\mu$l of vesicles were transferred to the 2.4 ml cuvette with magnetic stirring, and the internal pH recovery was continuously recorded with a DAT tape recorder and displayed on a penwriter. The experiments showed that the giant sarcolemmal vesicles contained esterase activity sufficient to accumulate measurable amounts of the BCECF form from the low external BCECF-AM concentration used in the present study. The $pH_i$ recovery rate due to bicarbonate-dependent anion exchange could not be measured, because constant pH in the cuvette could not be obtained in the presence of the $HCO_3^-/CO_2$ buffer system.

### 2.5. Buffer capacity

The vesicles were lysed with the detergent deoxycholate, and the solution titrated with HCl/NaOH. The water space was determined with labelled lactate.

### 2.6. Statistics

A two-tailed *t*-test for non paired observations was used.

## 3. Results

### 3.1. Buffer capacity

The mean buffer capacity in mixed vesicles measured with HCl/NaOH titration was 41.1 ± 6.7 meq./l of vesicular water space/pH unit ($n = 6$) in the pH interval 6.5–7.4.

### 3.2. $Na^+$ influx experiments

The $Na^+/H^+$-dependent $Na^+$ flux into mixed vesicles was defined as the difference between influx with and without 1 mM amiloride and in the presence of an inward directed $Na^+$ gradient of 85 mM. In addition, the influx was measured both in the presence of a pH gradient ($pH_o = 7.4$, $pH_i = 6.5$) and without a pH gradient ($pH_o = pH_i = 7.4$) (Table 1). The $Na^+$ influx in the presence of the pH gradient was 85% higher than without the gradient. Amiloride inhibited total $Na^+$ uptake by approx. 50%.

### 3.3. Lactate efflux experiments

Lactate efflux from mixed vesicles was measured with 40 mM lactate and zero *trans* conditions at pH 7.4 and 6.5 (Table 1). The carrier-mediated lactate efflux was defined as the fraction of the total lactate efflux, which could not be inhibited by 250 $\mu$M quercetin [26]. The non-carrier-mediated fraction (which is expected to be diffusion of undissociated lactic acid) was higher at $pH_i$ 6.5 than at $pH_i$ 7.5 in agreement with the higher concentration of undissociated lactic acid at low pH. On the contrary, the carrier-mediated lactate flux was lower at low pH.

### 3.4. $HCO_3^-/Cl^-$ exchange

The $HCO_3^-/Cl^-$ exchange in mixed vesicles was quantified from the $Cl^-$ efflux with and without $HCO_3^-$, and in

Table 1
Capacity to remove $H^+$ in vesicles from mixed muscles

| | |
|---|---|
| *$Na^+/H^+$ exchange (nmol $Na^+$/mg protein per min)* | |
| Amiloride-sensitive Na uptake, pH gradient present | 33.2 ± 9.5 ($n = 9$) |
| Amiloride-sensitive Na uptake, no pH gradient | 17.8 ± 6.4 ($n = 9$) |
| *Lactate/$H^+$ co-transport, efflux (nmol lac/mg protein per min)* | |
| Total efflux at $pH_i$ 7.4 and $pH_o$ 7.4 | 216 ± 37 ($n = 6$) |
| Carrier-mediated efflux at $pH_i$ 7.4 | 117 ± 35 ($n = 6$) |
| Total efflux at $pH_i$ 6.5 | 240 ± 33 ($n = 6$) |
| Carrier-mediated efflux at $pH_i$ 6.5 | 85 ± 11 ($n = 6$) |
| *$Cl^-/HCO_3^-$ exchange (nmol $Cl^-$/mg protein per min)* | |
| Total $HCO_3^-$-dependent $Cl^-$ efflux | 62.2 ± 14.9 ($n = 13$) |
| DIDS-sensitive $Cl^-$ efflux | 46.5 ± 9.0 ($n = 10$) |

Mean ± S.D. shown. $n$ = number of experiments.
Carrier mediated lactate flux was defined as the fraction which could be inhibited by 250 $\mu$M quercetin.
Inhibitors used: DIDS; 0.2 mM. Amiloride; 1 mM.

Table 2
$pH_i$ recovery capacity of the lactate/$H^+$ and $Na^+/H^+$ systems in vesicles from mixed muscles

| System | Initial $pH_i$ recovery (pH units/min) |
|---|---|
| *Lactate-$H^+$ dependent recovery* | |
| 40 mM lactate present | $0.332 \pm 0.072$ ($n = 7$) |
| 40 mM lactate + inhibitor (quercetin) | $0.152 \pm 0.030$ ($n = 7$) |
| *$Na^+/H^+$-exchange dependent recovery* | |
| EIPA-sensitive rate, $pH_i$ 6.5 | $0.067 \pm 0.004$ ($n = 6$) |

Recovery rate at pH 6.5 was calculated from the $pH_i$ increase within the first 30 s after the vesicles were transferred to the cuvette. Mean ± S.D. $n$ = number of experiments. The effect of EIPA was defined as the difference in $pH_i$ recovery with and without 10 μM of the inhibitor.

other experiments from the effect of DIDS in the presence of $HCO_3^-$. 71% of the $HCO_3^-$- induced $Cl^-$ efflux could be inhibited with 0.2 mM DIDS (Table 1).

### 3.5. Lactate-$H^+$ co-transport-dependent $pH_i$ recovery

Internal pH recovery was measured in mixed vesicles with the BCECF method in the presence of internal lactate and zero trans conditions. The vesicles were preincubated with or without 40 mM lactate at pH 6.5. In some experiments carrier-mediated flux was inhibited with 250 μM quercetin [26]. The initial lactate-dependent $pH_i$ recovery rates were defined from the initial $pH_i$ recovery rate with and without lactate present (Table 2). At pH 6.5 the inhibitor-sensitive part of the efflux made up one third of the total flux.

### 3.6. $Na^+/H^+$-exchange-dependent $pH_i$ recovery

Internal pH in mixed vesicles was acidified with the $NH_4^+$ prepulse method. Incubation with 40 mM $NH_4Cl$ was found to decrease $pH_i$ to 6.5. The initial $Na^+/H^+$-exchange-dependent fraction of $pH_i$ recovery was defined as the difference in recovery with and without 10 μM of the amiloride derivative ethyl isopropyl amiloride (EIPA) and in the presence of 15 mM $Na^+$ (Table 2).

Both the studies with isotopes (Table 1) and the pH measurements (Table 2) showed that at pH 6.5 the capacity of the $Na^+/H^+$ system made up 14–20% of the rate of the lactate-dependent $H^+$ efflux.

### 3.7. Fiber type specific transport capacity

Experiments were carried out with vesicles from mainly red and white muscle fibers. The fiber type specific activity of the three systems is shown in Table 3, where $HCO_3^-/Cl^-$ was defined from the DIDS-sensitive fraction of the $Cl^-$ flux, $Na^+/H^+$ defined from the amiloride-sensitive $Na^+$ flux, and lactate/$H^+$ was the total lactate-dependent flux (carrier-mediated + diffusion). The lactate/$H^+$ system and the bicarbonate-dependent systems had significantly higher capacities in vesicles from red compared to vesicles from white muscle, whereas the $Na^+/H^+$ system seems to be homogeneously distributed.

## 4. Discussion

During muscle activity lactic acid accumulates in cells and consequently cellular pH is decreased. After high intensity exhaustive exercise the lactate content may reach 40 mM and pH may decrease to 6.5. These values were therefore used as the starting points from which $pH_i$ recovery took place in the present model.

The main conclusion was that the capacity of the lactate-dependent transport mechanism to remove $H^+$ was approximately twice the sum of the $Na^+/H^+$ exchange and the $HCO_3^-/Cl^-$ exchange systems. In contrast to the present finding, it has been reported that lactic acid efflux is too slow to account for much proton efflux in recovery [15]. The calculations were, however, based on a maximal lactate efflux from human skeletal muscle of 0.7–2 mmol/l per min. Other studies with human skeletal muscle have reported lactate efflux values in the range 6–9 mmol/kg per min [11]. The contribution of the lactate/$H^+$ efflux in the former study was therefore underestimated.

The lactate/$H^+$ co-transport system operates stoichio-

Table 3
Transport capacities in vesicles from red and white muscle fibers

| Transport capacity (nmol/mg per min) | Red muscle fiber | White muscle fiber | Significance |
|---|---|---|---|
| $HCO_3^-$ / $Cl^-$ | $66.6 \pm 18.8$ ($n = 9$) [a] | $46.0 \pm 12.1$ ($n = 10$) [a] | $P < 0.02$ |
| $Na^+/H^+$ | $37.2 \pm 13.5$ ($n = 42$, $N = 6$) [b] | $35.7 \pm 14.7$ ($n = 38$, $N = 6$) [b] | not significantly different |
| $Lac^-/H^+$ | $281 \pm 22$ ($n = 8$) [c] | $207 \pm 21$ ($n = 8$) [c] | $P < 0.01$ |

Definitions: The $HCO_3^-/Cl^-$ capacity was defined from the DIDS-sensitive fraction of the total initial $Cl^-$ flux. The $Na^+/H^+$ capacity was defined from the amiloride-sensitive part of the initial $Na^+$ flux, and lactate/$H^+$ was the total initial lactate transport. The initial pH in all experiments was 6.5. Values are given as mean ± S.D.

[a] $n$ = number of series each with 8 samples.

[b] $N$ = number of preparations, $n$ = total number of single experiments.

[c] $n$ = number of series with 10 samples.

metrically in a 1:1 manner, which can be concluded from both the electroneutrality [22] and from simultaneous measurements of lactate and $H^+$ influx [12]. Consequently the measurement of lactate fluxes (Table 1) also represent parallel $H^+$ fluxes. In addition, the lactate-dependent $pH_i$ recovery was quantified from pH measurements. Because of the electroneutral nature of the transport systems studied, the lack of a membrane potential in the vesicles was considered not to have interfered with the measurements.

The $Na^+/H^+$ exchange was quantified both from the $pH_i$ recovery rates and from the amiloride-sensitive $Na^+$ influx in the presence of 100 mM external $Na^+$, which is sufficient to saturate the system [7]. Since the exchange system operates in a 1:1 manner [7] the $Na^+$ fluxes were equivalent to oppositely directed $H^+$ fluxes. Because other systems, for instance the amiloride insensitive Na,K,2Cl co-transport [9] could mediate a $Na^+$ flux, only the amiloride-sensitive flux can be taken as an indication of the activity of the $Na^+/H^+$ system. Although different techniques were used both the $pH_i$ recovery rates (Table 2) and the $Na^+$ influx experiments (Table 1) showed that the capacity of the amiloride-sensitive $Na^+/H^+$ exchange to remove $H^+$ is small (20% and 14%, respectively) compared to the capacity of the total lactate-dependent transport. In the present study the $Na^+/H^+$ system was active at pH 7.4, which is in agreement with most other studies of skeletal muscle, whereas in most other tissues the $Na^+/H^+$ exchanger is inactive at pH 7.4 [7]. In contrast, Grossie et al. [8] reported that the exchange system is not active for $pH_i$ higher than 7.1.

In the $HCO_3^-$ experiments $Na^+$ was also present, therefore the measurements account for both the DIDS-sensitive $Na^+$- independent $HCO_3^-/Cl^-$ exchange and for a DIDS-sensitive $Na^+$-dependent system, if present [21]. The initial $Cl^-$ efflux could be used to quantify the $HCO_3^-$ influx because of the 1:1 nature of the system. Because other systems, for instance the DIDS insensitive Na,K,2CL co-transport system [9] can mediate a $Cl^-$ flux, only the DIDS-sensitive component was used in the calculations. If the DIDS-sensitive fluxes are used as an indication of the capacity of the $HCO_3^-/Cl^-$ exchange system to remove $H^+$, it can be calculated that this system is equivalent to 140% of the capacity of the $Na^+/H^+$ system.

### 4.1. Stoichiometry of total $H^+$ and lactate removal

Measurements of lactate and $H^+$ fluxes from the cells in the recovery period after muscle activity and measurements of the lactate and acid deposition in the extracellular space, have shown that the $H^+$ efflux in most studies exceeds the lactate efflux rate [4,16–18,24]. Only the lactate/$H^+$ carrier and simple diffusion of lactic acid mediate lactate efflux, whereas also the $Na^+/H^+$ exchange and bicarbonate-dependent exchange mediate $H^+$ removal. The total capacity to remove $H^+$ therefore exceeds the capacity to transport the lactate ion. At $pH_o$ 6.5, with 40 mM lactate and based on studies with vesicles from mixed muscles (Tables 1 and 2) the removal ratio ($H^+$/lactate) is 1.3. This is close to the ratio of 1.56 obtained with the NMR-technique and human muscles [18]. This ratio is not expected to be constant, although always higher than 1, because the concentration- and pH-dependency of the involved transport systems are different. In addition, it has been shown in rats that the activity of the lactate/$H^+$ co-transport system is dependent on fiber type and age of the animal [13] and can be increased by training [19]. Also of importance is that the $Na^+/H^+$ system is hormone-sensitive and the relative activity of the $Na^+/H^+$ system and the $HCO_3^-$-dependent system is dependent on fiber type.

### 4.2. Fiber type-specific distribution of transport systems

It is not possible to obtain pure red and white muscle preparations from the rat. Based on muscle area and fiber type distribution the fibers used to produce vesicles from 'red' fibers were calculated to consist of 52% SO (slow-twitch oxidative), 46% FOG (fast twitch oxidative-glycolytic) and 2% FG (fast-twitch glycolytic), while muscles used to produce vesicles from 'white' muscles consisted of 4% SO, 14% FOG and 82% FG [3].

The experiments with vesicles from red and white muscles showed no fiber type specific distribution of the $Na^+/H^+$ system. It must be noted that the present technique only quantified the basal activity of the system, a hormonal induced activation of the system is probably lost during the preparation technique.

The activity of the $HCO_3^-/Cl^-$ system was significantly higher in vesicles from red compared to white fibers. It has been reported for mouse soleus (mainly slow twitch fibers) that the capacity of the $HCO_3^-$-dependent system is 20% of the $Na^+/H^+$ system [1], whereas for mouse extensor digitorum longus muscles (fast twitch fibers) the two transport systems possess nearly the same capacity [8]. This discrepancy could be due to species specific differences in transport system distribution.

The experiments confirmed that the total lactate transport is higher in vesicles from red than from white rat fibers [13].

In summary, it can be seen that the total capacity to remove protons and lactate is higher in red compared to white muscle fibers. This finding seems surprising since the red fibers are less glycolytic than the white fibers, which possess the highest lactate production capacity. The high activity of the lactate/$H^+$ system and the bicarbonate-dependent system could however be an adaptation to the recruitment pattern, since the red fibers are usually active for longer time periods. In addition, the high transport capacities in red fibers could contribute to the higher fatigue resistance compared to white fibers. Alternatively, the high activity of the lactate/$H^+$ carrier in red fibers

could ensure a high uptake rate, which is consistent with the high capacity for lactate metabolism (oxidation) found in these fibers.

### 4.3. pH regulation

The three transport systems quantified in the present report all participate in pH regulation in muscle cells. However, the systems differ in the way they are activated. The lactate/$H^+$ co-transport system, which possesses a high capacity, is driven by the gradient for lactate, which is transported downhill. This system can therefore only operate if lactic acid is produced in the cells. It can be seen from Table 1 that the carrier-mediated lactate flux is decreased at low pH, which seems inappropriate, since low pH is associated with a high lactate accumulation. The simple diffusion of undissociated lactic acid, on the contrary, is increased at low pH because of the higher concentration of the undissociated form, which will also increase $pH_i$ recovery [12,14].

The $HCO_3^-/Cl^-$ system(s) is dependent on the distribution of $HCO_3^-$ and $Cl^-$, which is coupled to the $Na^+$ gradient [9]. The $Na^+/H^+$ exchange system is also dependent on the $Na^+$ gradient, and is strongly activated by hormones and an internal pH decrease [7]. The steep $pH_i$ dependence of the $Na^+/H^+$ exchange system ensures that the system responds to small fluctuations in $pH_i$. The $Na^+/H^+$ system is therefore very suitable for the adjustments of $pH_i$ at near resting conditions, whereas the low capacity implies that the system is of minor importance for $H^+$ efflux in periods with a high lactic acid production.

## Acknowledgements

This study was supported by The Danish Natural Research Council, and The Danish National Research Foundation (504-14).

## References

[1] Aickin, C.C. and Thomas, R.S. (1977) J. Physiol. 273, 295–316.
[2] Aickin, C.C. (1986) Annu. Rev. Physiol. 48, 349–361.
[3] Armstrong, R.B. and Phelps, R.O. (1984) Am. J. Anat. 171, 259–272.
[4] Barbee, R.W., Stainsby, W.N. and Chirtel. S.J (1983) J. Appl. Physiol. 54(6), 1687–1692.
[5] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[6] Fliegel, L. and Fröhlich, O. (1993) Biochem. J. 296, 273–285.
[7] Frelin, C., Vigne, P., Jean, T., Barbry, P. and Lazdunski, M. (1989) in $Na^+/H^+$ Exchange (Grinstein, S., ed.), CRC Press, Boca Raton, Florida.
[8] Grossie, J., Collins, C. and Julian, M. (1988) J. Membr. Biol. 105, 265–272.
[9] Hoffmann, E.K. and Simonsen, L.O. (1989) Physiol. Rev. 69(2), 315–382.
[10] Juel, C. (1988) Acta Physiol. Scand. 132, 363–371.
[11] Juel, C., Bangsbo, J., Graham, T. and Saltin, B. (1990) Acta Physiol. Scand. 140, 47–159.
[12] Juel, C. (1991) Biochim. Biophys. Acta 1065, 15–20.
[13] Juel, C., Honig, A. and Pilegaard, H. (1991) Acta Physiol. Scand. 143, 361–365.
[14] Juel, C., Kristiansen, S., Pilegaard, H., Wojtaszewski, J. and Richter, E.A. (1994) J. Appl. Physiol. 76(3), 1031–1036.
[15] Kemp, G.J., Taylor, D.J., Styles, P. and Radda, G.K. (1993) NMR Biomed. 6, 73–83.
[16] Kowalchuk, J.M., Heigenhauser, G.J.F., Lindinger, M.I., Sutton, J.R. and Jones, N.L. (1988) J. Appl. Physiol. 65(5), 2080–2089.
[17] Medbø, J.I. and Sejersted, O.M. (1985) Acta Physiol. Scand. 125, 97–109.
[18] Pan, J.W., Hamm, J.R., Hetherington, H.P., Rothman, D.L. and Shulman, R.G. (1991) Magnet. Resonance Med. 20, 57–65.
[19] Pilegaard, H., Juel, C. and Wibrand, F. (1993) Am. J. Physiol. 264, E156–E160.
[20] Ploug, T., Wojtaszewski, J., Kristiansen, S., Hespel, P., Galbo, H. and Richter, E.A. (1993) Am. J. Physiol. 264, 270–E278.
[21] Putnam, R.W. (1990) Am. J. Physiol. 258, C470–C479.
[22] Roth, D.A. and Brooks, G.A. (1990) Arch. Biochem. Biophys. 279, 377–385.
[23] Ross, A. (1975) J. Physiol. 249, 1–25.
[24] Sahlin, K., Harris, R.C., Nylind, B. and Hultman, E. (1976) Pflügers Arch. 367, 143–149.
[25] Watt, P.W., MacLennan, P.A., Hundal, H.S., Kuret, C.M. and Rennie, M.J. (1988) Biochim. Biophys. Acta 944, 213–222.
[26] Wibrand, F. and Juel, C. (1994). Biochem. J. 299, 533–537.